

**WHAT IS CLAIMED IS:**

1. A method of infecting a neoplasm in a mammal with a virus comprising  
5 administering an interferon-sensitive, replication-competent clonal RNA virus to said mammal.
2. A method of infecting a neoplasm in a mammal with a virus comprising administering  
a replication-competent clonal RNA virus to said mammal wherein said virus has  
10 sensitivity to interferon.
3. A method of treating a neoplasm in a mammal comprising administering to said  
mammal a therapeutically effective amount of an interferon-sensitive, replication-  
competent clonal RNA virus.  
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4. A method as in claim 1 wherein said RNA virus replicates at least 100-fold less in  
the presence of interferon compared to in the absence of interferon.
5. A method as in claim 1 wherein said RNA virus replicates at least 1000-fold less in  
20 the presence of interferon compared to in the absence of interferon.
6. A method as in claim 1 wherein said administering step is systemic.
7. A method as in claim 1 wherein said neoplasm is a cancer.
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8. A method as in claim 1 wherein said mammal is a human.
9. A method as in claim 1 wherein said clonal virus is plaque purified.

10. A method as in claim 1 wherein said clonal virus is of recombinant clonal origin.

11. A method as in claim 1 wherein said RNA virus is a Paramyxovirus.

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12. A method as in claim 11 wherein said Paramyxovirus is purified to a level of at least  $2 \times 10^9$  PFU per mg of protein.

10 13. A method as in claim 11 wherein said Paramyxovirus is purified to a level of at least  $1 \times 10^{10}$  PFU per mg of protein.

14. A method as in claim 11 wherein said Paramyxovirus is purified to a level of at least  $6 \times 10^{10}$  PFU per mg of protein.

15 15. A method as in claim 11 wherein said Paramyxovirus is purified to a level in which the particle per PFU ratio is no greater than 5.

16. A method as in claim 11 wherein said Paramyxovirus is purified to a level in which the particle per PFU ratio is no greater than 3.

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17. A method as in claim 11 wherein said Paramyxovirus is purified to a level in which the particle per PFU ratio is no greater than 1.2.

18. A method as in claim 11 wherein said Paramyxovirus is avian paramyxovirus type 2.

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19. A method as in claim 11 wherein said Paramyxovirus is NDV.

20. A method as in claim 11 wherein said Paramyxovirus is mumps virus.

21. A method as in claim 11 wherein said Paramyxovirus is human parainfluenza virus.

22. A method as claim 1 wherein said RNA virus is selected from the group consisting of a Rhabdovirus, Togavirus, Flavivirus, Reovirus, Picornavirus, and Coronavirus.

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23. A method as in claim 22 wherein said Togavirus is Sindbis virus.

24. A method as in claim 22 wherein said Reovirus has a modification at omega 3.

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25. A method as in claim 22 wherein said Reovirus has an attenuating mutation at omega 1.

26. A method as in claim 22 wherein said Reovirus is an attenuated rotavirus

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27. A method as in claim 26 wherein said rotavirus is rotavirus WC3.

28. A method of infecting a neoplasm in a mammal with a virus comprising administering an interferon-sensitive, replication-competent clonal vaccinia virus, having one or more mutations in one or more genes selected from the group consisting of K3L, E3L, and B18R, to said mammal.

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29. A method of infecting a neoplasm in a mammal with a virus comprising administering a replication-competent clonal vaccinia virus, having one or more mutations in one or more genes selected from the group consisting of K3L, E3L, and B18R, to said mammal wherein said virus has sensitivity to interferon.

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30. A method treating a neoplasm in a mammal comprising administering to said mammal a therapeutically effective amount of an interferon-sensitive, replication-competent clonal vaccinia virus, having one or more mutations in one or more genes

selected from the group consisting of K3L, E3L, and B18R.

31. A method as in claim 30 wherein said mammal is a human.

5 32. A method as in claim 30 wherein said vaccinia virus is a vaccinia virus having an attenuating mutation in a gene selected from the group encoding vaccinia growth factor, thymidine kinase, thymidylate kinase, DNA ligase, ribonucleotide reductase and dUTPase.

10 33. A method of infecting a neoplasm in a mammal with a virus comprising administering an interferon-sensitive, replication-competent clonal DNA virus, selected from the group consisting of Adenoviruses, Parvoviruses, Papovaviruses, and Iridoviruses, to said mammal.

15 34. A method of infecting a neoplasm in a mammal with a virus comprising administering a replication-competent clonal DNA virus, selected from the group consisting of Adenoviruses, Parvoviruses, Papovaviruses, and Iridoviruses, to said mammal wherein said virus has sensitivity to interferon.

20 35. A method of treating a neoplasm in a mammal comprising administering to said mammal a therapeutically effective amount of an interferon-sensitive, clonal DNA virus selected from the group consisting of Adenoviruses, Parvoviruses, Papovaviruses, and Iridoviruses.

25 36. A method as in claim 33 wherein said mammal is a human.

37. A method as in claim 33 wherein said Adenovirus virus has a modification in the VA1 transcripts causing said Adenovirus to become interferon-sensitive.

38. A method as in claim 37 wherein said Adenovirus virus is selected from the group consisting of vaccine strains of Ad-4, Ad-7 and Ad-21.

5 39. A method of infecting a neoplasm in a mammal with a virus comprising administering an interferon-sensitive, replication-competent clonal Herpesvirus to said mammal.

10 40. A method of infecting a neoplasm in a mammal with a virus comprising administering a replication-competent clonal Herpesvirus to said mammal wherein said virus has sensitivity to interferon.

15 41. A method treating a neoplasm in a mammal comprising administering to said mammal a therapeutically effective amount of an interferon-sensitive, replication-competent clonal Herpesvirus.

42. A method as in claim 41 wherein said Herpesvirus is a member of the subfamily Betaherpesvirus or subfamily Gammaherpesvirus.

20 43. A method as in claim 41 wherein said Herpesvirus is a member of the subfamily Alphaherpesvirus that is not HSV-1.

44. A method as in claim 41 wherein said mammal is a human.

25 45. A method as in claim 41 wherein said Herpesvirus is a member of the subfamily Alphaherpesvirus which has decreased expression of the (2'-5')An analog.

46. A method as in claim 45 wherein said Herpesvirus is a Herpesvirus having an attenuating mutation selected from the group consisting of the genes encoding

thymidine kinase, ribonucleotide reductase, or a deletion in the b'a'c' inverted repeat locus.

47. A method as in claim 45 wherein said Herpesvirus has a modification in the gamma  
5 34.5 gene.

48. A method as in claim 41 wherein said Herpesvirus has a modification in the gamma 34.5 gene and an attenuating mutation in the gene encoding of thymidine kinase, or a deletion in the b'a'c' inverted repeat locus or functionally analogous loci.

49. A method as in claim 41 wherein said Herpesvirus is a Herpesvirus having an attenuating mutation in a gene selected from the group consisting of thymidine kinase, and ribonucleotide reductase, or a deletion in the b'a'c' inverted repeat locus.

15 50. A method as in claim 1 wherein said neoplasm is a cancer selected from the group consisting of lung, colon, prostate, breast and brain cancer.

51. A method as in claim 1 wherein said neoplasm is a solid tumor.

20 52. A method as in claim 50 wherein said brain cancer is a glioblastoma.

53. A method as in claim 1 wherein said virus contains a gene encoding interferon to permit the viral expression of interferon.

25 54. A method as in claim 1 wherein said virus contains a gene encoding a pro-drug activating enzyme.

55. A method as in claim 1 further comprising administering IFN, before, during or after administration of said virus.



56. A method as in claim 55 wherein said interferon is selected from the group consisting of  $\alpha$ -IFN,  $\beta$ -IFN,  $\omega$ -IFN,  $\gamma$ -IFN, and synthetic consensus forms of IFN.

5 57. A method as in claim 1 further comprising administering a tyrosine kinase inhibitor before, during or after administration of said virus.

58. A method as in claim 1 further comprising administering a compound selected from the group of compounds comprising a purine nucleoside analog, tyrosine kinase  
10 inhibitor, cimetidine, and mitochondrial inhibitor.

59. A method as in claim 1 further comprising administering a chemotherapeutic agent before, during or after administration of said virus.

15 60. A method as in claim 1 further comprising administering a cytokine before, during or after administration of said virus.

61. A method as in claim 1 further comprising administering an immunosuppressant before, during or after administration of said virus.

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62. A method as in claim 1 further comprising administering a viral replication controlling amount of a compound selected from the group consisting of IFN, ribavirin, acyclovir, and ganciclovir.

25 63. A method as in claim 1 wherein said administration is intravenous or intratumoral.

64. A method of infecting a neoplasm which is at least 1 centimeter in size in a mammal with a virus comprising administering a clonal virus, selected from the group consisting

of(l) RNA viruses; (2) Hepadenavirus; (3) Parvovirus; (4) Papovavirus; (5) Herpesvirus; (6) Poxvirus; and (7) Iridovirus, to said mammal.

5 65. A method of treating a neoplasm in a mammal, comprising administering to said mammal a therapeutically effective amount of a clonal virus selected from the group consisting of(1) RNA virus; (2) Hepadenavirus; (3) Parvovirus; (4) Papovavirus; (5) Herpesvirus; (6) Poxvirus; and (7) Iridovirus, wherein said neoplasm is at least 1 centimeter in size.

10 66. A method as in claim 64, wherein said neoplasm is at least 300 mm<sup>3</sup> in volume.

67. A method as in claim 64, wherein said RNA virus is a Paramyxovirus.

68. A method as in claim 67, wherein said Paramyxovirus is NDV.

15 69. A method as in claim 64, wherein said mammal is a human.

70. A method as in claim 64, wherein said administration is intravenous or intratumoral.

20 71. A method as in claim 64, wherein said paramyxovirus is purified to a level of at least  $2 \times 10^9$  PFU per mg protein.

72. A method as in claim 68, wherein said NDV is mesogenic.

25 73. A method as in claim 65 wherein said neoplasm is cancerous.

74. A method of treating a tumor in a mammal, comprising administering to said mammal a therapeutically effective amount of an RNA virus cytotoxic to said tumor,



wherein said mammal has a tumor burden comprising at least 1.5% of the total body weight of said mammal.

75. A method as in claim 74, wherein said tumor does not respond to chemotherapy.

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76. A method of screening tumor cells or tissue freshly removed from the patient to determine the sensitivity of said cells or tissue to killing by a virus comprising subjecting a tissue sample to a differential cytotoxicity assay using an interferon-sensitive virus.

10 77. A method as in claim 76 further comprising the step of screening said cells or tissue for protein, or mRNA encoding protein, selected from the group consisting of p68 protein kinase, C-Myc, C-Myb, ISGF-3, IRF-1, IFN receptor, and p58.

78. A method for identifying a virus with antineoplastic activity in a mammal comprising:

15 a) using said test virus to infect i) cells deficient in an interferon-mediated antiviral activity, and ii) cells competent in an interferon-mediated antiviral activity, and  
b) determining whether said test virus kills said cells deficient in an interferon-mediated antiviral activity preferentially to said cells competent in interferon-mediated antiviral activity.

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79. A method as in claim 78 wherein said cells deficient in an interferon-mediated antiviral activity are KB human head and neck carcinoma cells.

80. A method as in claim 78 wherein said cells competent in an interferon-mediated  
25 antiviral activity are human skin fibroblasts.

81. A method of making viruses for use in antineoplastic therapy comprising:

(a) modifying an existing virus by diminishing or ablating a viral mechanism for the inactivation of the antiviral effects of IFN, and optionally (b) creating an attenuating

mutation

- 5 82. A method of controlling viral replication in a mammal treated with a virus selected from the group consisting of RNA viruses, Adenoviruses, Poxviruses, Iridoviruses, Parvoviruses, Hepadnaviruses, Varicellaviruses, Betaherpesviruses, and Gammaherpesviruses comprising administering an antiviral compound.
83. A method as in claim 82 wherein said antiviral compound is interferon.
- 10 84. A method as in claim 82 wherein said antiviral is selected from the group consisting of ribavirin, acyclovir, and ganciclovir.
85. A method as in claim 82 wherein said antiviral is a neutralizing antibody to said virus.
- 15 86. A Paramyxovirus purified by ultracentrifugation without pelleting.
87. A Paramyxovirus purified to a level of at least  $2 \times 10^9$  PFU/mg protein.
- 20 88. A Paramyxovirus as in claim 87 wherein said paramyxovirus is grown in eggs and is substantially free of contaminating egg proteins.
89. A Paramyxovirus as in claim 87 wherein said paramyxovirus has a particle per PFU ratio no greater than 5.
- 25 90. A Paramyxovirus as in claim 87 wherein said paramyxovirus has a particle per PFU ratio no greater than 3.
91. A Paramyxovirus as in claim 87 wherein said paramyxovirus has a particle per PFU

ratio no greater than 1.2.

92. A Paramyxovirus purified to a level of at least  $1 \times 10^{10}$  PFU/mg protein.

5 93. A Paramyxovirus purified to a level of at least  $6 \times 10^{10}$  PFU/mg protein.

94. A Paramyxovirus as in claim 87 wherein said virus is cytotoxic.

10 95. A Paramyxovirus as in claim 87 wherein said Paramyxovirus is Newcastle disease virus.

96. An NDV as in claim 95 wherein said NDV is cytotoxic.

15 97. An NDV as in claim 95 wherein said NDV is mesogenic.

98. An RNA virus purified to a level of at least  $2 \times 10^9$  PFU/mg protein.

99. An RNA virus as in claim 98 wherein said virus is replication-competent.

20 100. A replication-competent cytotoxic virus which is interferon-sensitive and purified to a level of at least  $2 \times 10^9$  PFU/mg protein.

101. A cytotoxic virus as in claim 100 wherein said virus is clonal.

25 102. A cytotoxic DNA virus which is interferon-sensitive and purified to a level of at least  $2 \times 10^9$  PFU/mg protein.

103. A cytotoxic DNA virus as in claim 102 wherein said virus is a Poxvirus.

104. A Poxvirus as in claim 103 wherein said Poxvirus is a vaccinia virus having one or more mutations in one or more genes selected from the group consisting of K3L, E3L, and B18R.

5 105. A replication-competent vaccinia virus having a) one or more mutations in one or more of the K3L, E3L and B 1 8R genes, and b) an attenuating mutation in one or more of the genes encoding thymidine kinase, ribonucleotide reductase, vaccinia growth factor, thymidylate kinase, DNA ligase, dUTPase.

10 106. A replication-competent vaccinia virus having one or more mutations in two or more genes selected from the group consisting of K3L, E3L, and B18R.

107. A Herpesvirus having a modification in the expression of the (2'-5')A analog.

15 108. A Reovirus having a mutation at omega 3 and purified to a level of at least  $2 \times 10^9$  PFU/mg protein

109. A Reovirus having mutations at omega 1 and omega 3.

20 110. A method of purifying an RNA virus comprising the steps of:  
a) generating a clonal virus, and  
b) purifying said clonal virus by ultracentrifugation without pelleting.

111. A method as in claim 110 wherein said RNA virus is replication-competent.

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112. A method of purifying a Paramyxovirus comprising purifying said virus by ultracentrifugation without pelleting.

113. A method as in claim 112 wherein said purifying step additionally comprises prior

to said ultracentrifugation:

- a) plaque purifying to generate a clonal virus,
- b) inoculating eggs with said clonal virus,
- c) incubating said eggs,
- 5 d) chilling said eggs,
- e) harvesting allantoic fluid from said eggs and,
- f) removing cell debris from said allantoic fluid.

114. A method as in claim 112 wherein said Paramyxovirus virus is NDV.

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115. A method of infecting a neoplasm in a mammal with a virus comprising administering an interferon-sensitive, replication-competent RNA virus to said mammal.

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116. A method as in claim 1 wherein said virus is selected from the group consisting of the Newcastle disease virus strain MK1 07, Newcastle disease virus strain NJRoakin, Sindbis virus, and Vesicular stomatitis virus.

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117. A method of infecting a neoplasm in a mammal with a virus comprising administering a clonal virus selected from the group consisting of the Newcastle disease virus strain MK107, Newcastle disease virus strain NJRoakin, Sindbis virus, and Vesicular stomatitis virus.

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118. A method as in claim 1 or claim 28 or claim 33 wherein said virus is administered as more than one dose.

119. A method as in claim 118 wherein the first dose is a desensitizing dose.

120. A method as in claim 119 wherein said first dose is administered intravenously and a subsequent dose administered intravenously.

121. A method as in claim 119 wherein said first dose is administered intravenously and a subsequent dose administered intraperitoneally.

5 122. A method as in claim 119 wherein said first dose is administered intravenously and a subsequent dose administered intra-arterially.

123. A method of treating a neoplasm in a mammal comprising subjecting a sample from said mammal to an immunoassay to detect the amount of virus receptor present,  
10 and if said receptor is present, administering an interferon-sensitive, replication competent clonal virus, which bind said receptor, to said mammal.

124. A method as in claim 123 wherein said virus is Sindbis and said receptor is the high affinity laminin receptor.

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125. A method as in claim 1 or claim 28 or claim 33 wherein said virus is administered over the course of at least 4 minutes.

126. A method of treating tumor ascites comprising administering an interferon-  
20 sensitive, replication competent clonal virus.

127. A method of reducing pain in a mammal comprising administering an interferon-sensitive, replication competent clonal virus.